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(54) Title: LABELLED TAXANES

(57) Abstract: There are provided stable labelled taxanes, characterized in that the isotopes are present in the benzoyloxy group at the C-2 position. More particularly, one or more of the hydrogen or carbon atoms of the benzoyl residue at the C-2 position of the taxane skeleton is replaced respectively from deuterium or 13C.A process for their preparation and their use as internal standard in analytical methods for determining taxanes, useful anticancer drugs, are also provided.

LABELLED TAXANES

BACKGROUND OF THE INVENTION

Paclitaxel is a natural taxane diterpenoid, which was isolated from the bark of the Pacific Yew tree [E.Baloglu, D.G.I.Kingston; *J. Nat. Prod.* (1999) **62**: 1448-72]. The structure of the compound was first elucidated and published in 1971 [M.C.Wani, H.L.Taylor, M.E.Wall, P.Coggen, A.T.McPhail; *J. Am. Chem. Soc.* (1971) **93**: 2325-7]. Due to its unique ability to interfere with the tubulin system, which is crucial to the mitotic process of cells [S.B.Horowitz, J.Fant, P.B.Schiff; *Nature* (1979) **277**: 665], its formulated version in cremophor is currently in use for the treatment of several human cancers. Other paclitaxel analogues, herein called taxanes, are under development or are already used as cytotoxic agents. Primary among these taxanes is docetaxel. As a consequence there is a considerable need of analytical methods to determine taxanes and their metabolites in complex biological samples (e.g. animal and human plasma, urine, bile and tissues, in vitro cell culture media etc.). Several analytical methods have been published for the determination of paclitaxel or taxanes in biological fluids. Anyway most of them are not very convenient for daily routine analysis requiring multi-steps sample preparation. A significant improvement was obtained by using automated sample handling procedure followed by liquid chromatography (LC) and mass spectrometry detection (MS). One crucial aspect of a reliable and validated analytical method is the availability of a suitable internal standard. The addition of known amount of an internal standard to the unknown sample is a well-known and widely used procedure that can compensate for losses of the compound of interest during sample workup. According to this approach, any loss of the compound of interest can be determined by the loss of an equivalent fraction of internal standard. The precision and accuracy of this approach is strongly dependent on the structural similarity between the compound of interest and the internal standard. As a consequence it is generally agreed that the stable isotopically labelled analogues with the same molecular structure of a compound are the best internal standards for liquid chromatography-mass spectrometry (LC-MS) assay. In addition the internal standard should have preferably a molecular weight at least three mass units higher than that of the non-labelled compound of interest. Therefore there is a considerable need of paclitaxel, docetaxel or other taxanes

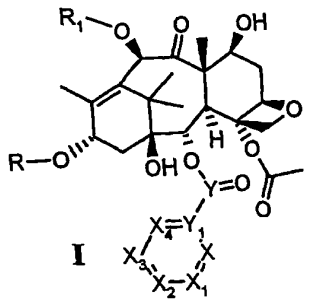
labelled with a high amount of stable isotopes in the skeleton in order to improve the accuracy and specificity of the analytical method to determine the drug or its metabolites, preferably in the biological fluids.

The chemistry of paclitaxel and its analogues is not trivial. In the literature, many papers reported methods that can be used to introduce stable- or radio-isotopes in the molecule of paclitaxel or its analogues [D.Giribone, E.Fontana, J. Labelled Compounds and Radiopharmaceuticals, 2000, 43, p. 933-41; I.Rodriguez et al., J. Labelled Compounds and Radiopharmaceuticals, 2000, 43, p. 169-76; D.D.Dischino et al., J. Labelled Compounds and Radiopharmaceuticals, 1995, 39, p. 173-9; S.deSuzzoni et al., J. Labelled Compounds and Radiopharmaceuticals, 1995, 36, p. 739-43; D.G.Walker et al., J. Labelled Compounds and Radiopharmaceuticals, 1995, 36, p. 479-91; D.G.Walker et al., J. Labelled Compounds and Radiopharmaceuticals, 1994, 34, p. 973-80; D. G. I. Kingston et al., J. Nat. Prod., 2000, 63, p. 726-34 and WO01/94328-A1]. These approaches involve the introduction of the isotopes in one of the side chains attached at taxane skeleton, mostly in that at the C-13 position. However these methods require the availability of synthons that can be prepared only according to non-trivial, time-consuming, multi-step synthetic sequences. Another possibility, valid only for paclitaxel, was the deuterium labelling at the 10-O-acetyl moiety, which was described in two papers [S.H.Hoke et al., J. Nat. Prod., 1994, 57, p. 277-86; P.Heinstein et al, J. Chem. Soc. Perkin Trans.1, 1996, p. 845-51]. In both cases the key-step was the reaction of the deuterated acetyl chloride with a suitable 10-O-deacetyl paclitaxel. Again the precursor is not easily available, the yield is low and the isotopic enrichment of the final compounds is questionable.

DETAILED DESCRIPTION

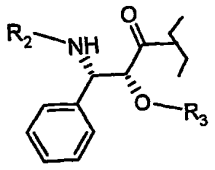
The present invention provides stable labelled paclitaxel, docetaxel or other taxanes, their use in analytical methods and a process for their preparation starting from commercially available stable labelled precursors at high isotopic enrichment and non-labelled taxane intermediates that can be easily synthesized starting from paclitaxel, docetaxel or other taxanes.

In particular, the present invention provides stable labelled taxanes of the formula I:



I

wherein R_1 represents hydrogen atom, an acetyl group or a hydroxy protecting group; R represents hydrogen atom, a hydroxy protecting group or a residue of the formula II:



II

wherein R_2 represents benzoyl or tert-butoxycarbonyl group; R_3 represents hydrogen atom or a hydroxy protecting group;

X, X_1, X_2, X_3 and X_4 independently represent CD, ^{13}CD, ^{13}CH or CH; Y and Y_1 independently represent C or ^{13}C, with the proviso that X, X_1, X_2, X_3, X_4 are not all CH when Y and Y_1 are both C.

The hydroxy protecting groups are preferably selected from silyl groups, such as trimethylsilyl, triethylsilyl, tert-butyldimethylsilyl or siamyldimethylsilyl group and 2,2,2-trichloroethoxycarbonyl group.

Preferably, R_1 represents hydrogen atom or an acetyl group, R represents a residue of the formula II wherein R_2 represents benzoyl or tert-butoxycarbonyl group and R_3 represents hydrogen atom; X, X_1, X_2, X_3, X_4 are ^{13}CH, Y and Y_1 are both C.

The preferred compounds according to the present invention are:

a) 2-[ring-U-^{13}C]-benzoyl-paclitaxel;

b) 2-[ring-U-^{13}C]-benzoyl-docetaxel and

c) 2-[ring-U-^{13}C]-benzoyl-10-desacetyl-baccatin III,

more preferably with an isotopic enrichment of at least 95%, still more preferably greater than 99%.

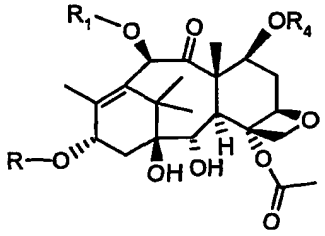
Another object of the present invention is the use of a labelled taxane of the formula I as defined above in an analytical method, preferably in order to determine a taxane in the biological fluids.

More preferably, the present invention provides the use of a labelled taxane of the formula I as defined above as an internal standard.

Still more preferably, the present invention provides the use of 2-[ring-U-^{13}C]-benzoyl-paclitaxel as an internal standard in an analytical method for determining paclitaxel in the biological fluids.

The present invention also provides a process for preparing the labelled compounds of the formula I, which process comprises:

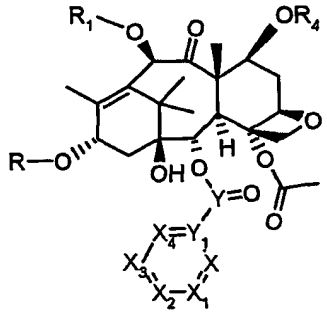
i) reacting a compound of the formula III



III

wherein R_4 represents a hydroxy protecting group and R, R_1 are as above defined, with an isotopically labelled benzoic acid,

ii) removing of the hydroxy protecting groups from the resultant compound of the formula IV:



IV

wherein R, R_1, R_4, X, X_1, X_2, X_3, X_4, Y and Y_1 are as above defined, to give the desired labelled taxanes of formula I and, if desidered,

iii) converting a compound of formula I into a different compound of formula I

The step i) is performed with an isotopically labelled benzoic acid (for example [ring-U-^{13}C]benzoic acid) in the presence of a condensing agent (for example N,N'-dicyclohexyl-carbodiimide) and a catalyst if needed (for example 4-pyrrolidinopyridine).

This reaction is carried out under inert atmosphere, for example under nitrogen, in an inert organic solvent such as aromatic hydrocarbons, for example toluene, and at elevated temperature, for example 60°C.

The molar ratio of the labelled benzoic acid and the compounds of formula III is 1÷20 to 1, preferably 5÷10 to 1. The molar ratio of the labelled benzoic acid and the condensing agent is preferably 1 to 1. The molar ratio of the catalyst, if present, and the labelled benzoic acid is about 1 to 15. The progress of this reaction is checked by an analytical method, for example thin layer chromatography or high performance liquid chromatography or mass spectrometry, and is complete when no starting material of formula III is detected, generally within about 28 hours. At the end of the reaction the mixture is preferably diluted with a solvent which does not dissolve the reaction byproducts, and filtered, for example through a sintered glass filtering funnel, to remove the solid materials. The crude material containing the compounds of formula IV is recovered after solvent evaporation to dryness.

The crude material containing the compounds of formula IV is preferably purified, before the subsequent step ii), by using techniques well known in the art. For example, preparative-column chromatography using silica gel along with appropriate eluants as organic solvents may be used to effectively purify the desired compound so as the following cleavage reaction is successfully carried out.

The step ii) is carried out by cleavage of the oxygen-protecting group bonds of the compounds of formula IV. The cleavage of the silicon-oxygen bonds of the silyl protected hydroxyl groups can be accomplished for example by means of acidic treatment. On the other hand the cleavage of the carbon-oxygen bond of the acetylated hydroxyl groups can be accomplished for example by means of a base mediated peroxide action. The cleavage of the silicon-oxygen bonds of the silyl protected hydroxyl groups is preferably carried out under inert atmosphere, for example under nitrogen, in an anhydrous polar protic organic solvent such as alkanols, for example methanol, and at room temperature, for example 25°C. The concentration of the acid is

preferably within the range of 1 – 2 M. The progress of this reaction is checked by an analytical method, for example thin layer chromatography or high performance liquid chromatography or mass spectrometry, and is complete when no starting material of formula IV is detected, generally within about 1 hour.

At the end of this reaction the mixture is diluted with a solvent non-miscible with water, for example ethyl acetate, then washed first with a solution of a base, for example sodium bicarbonate in water and eventually with a saturated water solution of an inorganic salt, for example sodium chloride.

The organic solution of the compound of interest is dried and filtered, for example through phase separator membrane or a sintered glass filtering funnel with an inorganic drying agent such as sodium sulfate, and the crude material containing compound I is recovered after solvent evaporation to dryness.

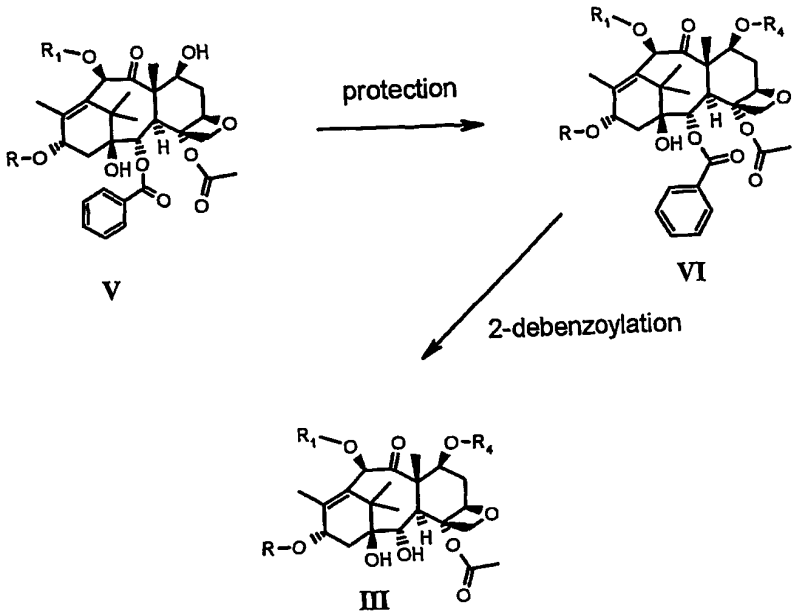
The crude material containing the compounds of formula I is preferably purified by using techniques well known in the art.

For example, preparative-column chromatography using C-8 reverse phase HPLC along with appropriate eluants as mixtures of water and organic solvents may be used to effectively purify the desired compound. The pure compound I is recovered as an organic solvent solution by partitioning the HPLC eluate of interest between a solvent non-miscible with water, for example ethyl acetate, and a saturated water solution of an inorganic salt, for example sodium chloride.

The pure the compounds of formula I is recovered as a white solid after solvent evaporation to dryness.

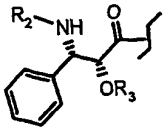
A compound of formula I wherein R represents hydrogen atom or a hydroxy protecting group, namely a labelled baccatin derivative, may be easily converted into a compound of formula I wherein R represents a residue of the formula II as defined above by well known procedures. In particular, we refer to the process described in WO97/42167-A.

The starting compounds of formula III can be easily obtained according to techniques well known in the art, summarized in the following scheme, where R, R_1 and R_4 are as defined above:



V VI III

The compounds of formula III in which for example R_1 is an acetyl group, R_4 is a triethylsilyl group and R is a residue of formula II:



wherein R_2 is a benzoyl or tert-butoxycarbonyl group and R_3 is a tert-butyldimethylsilyl group can be obtained starting from paclitaxel or docetaxel according to techniques well known in the art [see for example H.Park et al. J. Med. Chem., 1996, 39, p. 2705-2709; D.G.I.Kingston et al., J. Med. Chem., 1998, 31, p. 3715-3726; G.I.Georg et al., Tetrahedron Letters, 1994, 35, p. 8931-8934]. For example, a selective 2-O-benzoyl cleavage can be accomplished by treatment of a dichloromethane solution of a compound of formula VI with a solution of benzyltrimethylammonium hydroxide in methanol (Triton B®) at low temperature in nitrogen atmosphere under strictly controlled conditions. The compounds of formula III in which for example R and R_4 are triethylsilyl groups and R_1 is an acetyl or triethylsilyl group can be obtained starting from baccatin or 10-deacetylbaccatin III according to techniques well known in the art [see for example S-H.Chen et al., Bioorg. Med. Chem. Letters, 1998, 8, p. 2227-2230; I.Ojima et al., Bioorg. Med. Chem. Letters, 1999, 9, p. 3423-3428].

EXAMPLE 1

2'-O-(*tert*-butyldimethylsilyl)-2-[ring-U-^{13}C]-benzoyl-7-O-(triethylsilyl)paclitaxel

To a stirred suspension of [ring-U-^{13}C]benzoic acid (0.1982 g, 1.48 mmol) in anhydrous toluene (1.5 ml) at about 25°C, N,N'-dicyclohexylcarbodiimide (0.3085 g, 1.50 mmol) and 4-pyrrolidinopyridine (15 mg, 0.1 mmol) were added under nitrogen. After 5 minutes of stirring the compound 2'-O-(*tert*-butyldimethylsilyl)-2-debenzoyl-7-O-(triethylsilyl)paclitaxel [0.1445 g, 0.15 mmol, prepared starting from paclitaxel as described by H.Park, M.Hepperle, T.C.Boge, R.H.Himes, G.I.Georg, *J. Med. Chem.* (1996) **39**: 2705-9 and by D.G.I.Kingston, A.G.Chaudhary, M.D.Chordia, M.Gharpure, A.A.L.Gunatilaka, P.Giannakakou, Y.Q.Jiang, C.M.Lin, H.Hamel, B.H.Long, C.R.Fairchild, K.A.Johnston, *J. Med. Chem.* (1998) **31**: 3715-26] was introduced into the reaction flask. The suspension was kept at 60°C under nitrogen with stirring for 28 hours. The mixture, which showed the absence of the taxane starting material (checked by HPLC on C-8 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid from 75:25:0.1 to 10:90:0.1 by volume), was then cooled to about 25°C, diluted with ethyl acetate (15 ml) and filtered obtaining a clear solution. After solvent evaporation under reduced pressure a crude material containing the compound 2'-O-(*tert*-butyldimethylsilyl)-2-[ring-U-^{13}C]-benzoyl-7-O-(triethylsilyl)paclitaxel was recovered. The crude material was dissolved in a mixture of ethyl acetate and *n*-hexane (3:7 by volume, 5 ml) and chromatographed on a silica gel column (150×6.0 ID mm) using a solvent system made by mixture of ethyl acetate and *n*-hexane (3:7 by volume, 2.5 l) as eluant. Fractions of about 50 ml were collected and checked by HPLC on C-8 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid from 75:25:0.1 to 10:90:0.1 by volume. The fractions from number 13 to number 28 were pooled as necessary and evaporated to dryness. Two batches of the title compound consisting of 77 mg and 47 mg with a purity of about 90% and 60% respectively were recovered as white solids. The purity was assessed by HPLC (on C-8 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid from 75:25:0.1 to 10:90:0.1 by volume, linear gradient over 13 minutes and 8 minutes of isocratic elution, detection wavelenght = 240 nM), the retention time of title compound (Rt = 17.7 minutes) was the same as the retention time of an authentic non-labelled sample. The mass spectrum of the title

compound was recorded using the electrospray ionization technique (ESI) with positive ion detection. The ESI mass spectrum showed the protonated molecular ions at m/z 1088 amu and also another characteristic cluster at m/z 1105 amu ($M+NH_4^+$). The NMR spectrum recorded in $CDCl_3$ at 400 MHz showed the following signals expressed as chemical shifts (ppm): 8.27-8.38, m; 7.89-7.99, m; 7.67-7.83, m; 7.27-7.52, m; 7.03-7.09, d; 6.45, s; 6.22-6.30, m; 5.72-5.77, dd; 5.69, d; 4.96, dd; 4.68, d; 4.48, dd; 4.32, d; 4.22, d; 3.85, d; 2.58, m; 2.47-2.56, m; 2.33-2.46, m; 2.17, s; 2.05-2.15, m; 2.03, d; 1.87-2.02, m; 1.71, d; 1.69, s; 1.27, s; 1.22, s; 0.87-0.98, t; 0.80, s; 0.54-0.64, m; -0.02, s; -0.29, s.

EXAMPLE 2

Crude 2-[ring-U-^{13}C]-benzoyl-paclitaxel

The compound 2'-O-(*tert*-butyldimethylsilyl)-2-[ring-U-^{13}C]-benzoyl-7-O-(triethylsilyl)paclitaxel, prepared in EXAMPLE 1, (73 mg, about 90% pure) was dissolved under nitrogen in a cold (about 4°C) 1.5M solution of hydrogen chloride in anhydrous methanol (4 ml). After 5 minutes of stirring the clear solution was warmed to about 25°C and stirred for further 60 minutes. The reaction mixture that showed the absence of taxane starting material (checked by HPLC on C-8 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid from 75:25:0.1 to 10:90:0.1 by volume) was diluted with ethyl acetate (40 ml) and was washed 4 times with a 4% (by weight) aqueous solution of sodium bicarbonate (3 ml each time). All the aqueous washings were pooled and extracted with ethyl acetate (6 ml). All the organic phases were pooled, washed 4 times with water saturated with sodium chloride (3 ml each time), filtered through a phase separator septa and evaporated to dryness under reduced pressure. A crude material containing the compound 2-[ring-U-^{13}C]-benzoyl-paclitaxel, (also named [^{13}C6]paclitaxel, 67 mg about 60% pure) was recovered as a white solid. The purity was assessed by HPLC (on C-8 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid from 75:25:0.1 to 10:90:0.1 by volume, linear gradient over 13 minutes and 8 minutes of isocratic elution, detection wavelenght = 240 nM), the retention time of title compound (Rt = 17.7 minutes) was the same as of an authentic non-labelled sample. The mass spectrum of the title compound was recorded using the electrospray ionization technique (ESI) with positive ion detection. The ESI mass spectrum showed the protonated molecular ions at m/z 860

amu and also two other characteristic clusters at m/z 877 amu ($M+NH_4^+$) and 882 amu ($M+Na^+$).

EXAMPLE 3

Crude 2-[ring-U-^{13}C]-benzoyl-paclitaxel

The compound 2-[ring-U-^{13}C]-benzoyl-paclitaxel, prepared in EXAMPLE 1, (45 mg, about 60% pure) was dissolved under nitrogen in a cold (about 4°C) 1.5M solution of hydrogen chloride in anhydrous methanol (4 ml). After 5 minutes of stirring the clear solution was warmed to about 25°C and stirred for further 60 minutes. The reaction mixture that showed the absence of the taxane starting material (checked by HPLC on C-8 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid from 75:25:0.1 to 10:90:0.1 by volume) was diluted with ethyl acetate (40 ml) and was washed 4 times with a 4% (by weight) aqueous solution of sodium bicarbonate (3 ml each time). All the aqueous washings were pooled and extracted with ethyl acetate (6 ml). All the organic phases were pooled, washed 4 times with water saturated with sodium chloride (3 ml each time), filtered through a phase separator septa and evaporated to dryness under reduced pressure. A crude material containing the compound ([^{13}C6]paclitaxel, 42 mg about 45% pure) was recovered as a white solid. The purity was assessed by HPLC (on C-8 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid from 75:25:0.1 to 10:90:0.1 by volume, linear gradient over 13 minutes and 8 minutes of isocratic elution, detection wavelenght = 240 nM), the retention time of title compound (Rt = 17.7 minutes) was the same as of an authentic non-labelled sample. The mass spectrum of the title compound was recorded using the electrospray ionization technique (ESI) with positive ion detection. The ESI mass spectrum showed the protonated molecular ions at m(z 860 amu and also two other characteristic clusters at m/z 877 amu ($M+NH_4^+$) and 882 amu ($M+Na^+$).

EXAMPLE 4

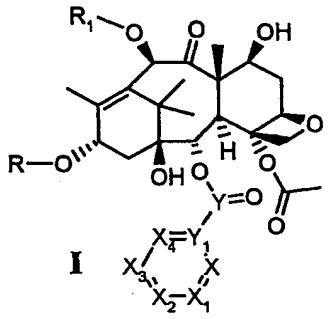
Purification of crude material containing 2-[ring-U-^{13}C]-benzoyl-paclitaxel

A solution of the crude material prepared in EXAMPLE 2 or in EXAMPLE 3 having the concentration of about 7 mg/ml was prepared in the solvent system dimethylsulphoxide-water-acetonitrile (25:25:50 by volume). Aliquots of 1÷4 ml of this

solution were injected in the following preparative-high performance liquid chromatography system. Column = Symmetry Prep C8 (mm 150 x 19 ID, 7μm particle size); mobile phase A = Water-acetonitrile 75:25 (by volume); mobile phase B = water-acetonitrile 10:90 (by volume); Elution = linear gradient from 100% of mobile phase A to 100% of mobile phase B over 13 minutes then isocratic at 100% of mobile phase B for 8 minutes; flow rate = 20 ml/minute; on line detection = UV (wavelength: 254 nm, sampling rate at least 2 points/second); fraction collection = manual triggering of fraction collector. The real time UV-absorbance plots of the runs were followed by sight so as to identify the peaks of the compound 2-[ring-U-^{13}C]-benzoyl-paclitaxel and to collect the eluates corresponding to the pure product. All the collected fractions containing the pure compound 2-[ring-U-^{13}C]-benzoyl-paclitaxel were pooled and concentrated to about one half of the original volume by rotary evaporation under reduced pressure. The solution was transferred into a separating funnel, added with one volume of water saturated with sodium chloride and extracted 4 times with ethyl acetate (6 ml each time). All the organic phases were pooled, filtered through phase separator septa and evaporated to dryness from *n*-hexane. The compound 2-[ring-U-^{13}C]-benzoyl-paclitaxel was obtained >98% chemically pure as a white solid. The purity was assessed by HPLC (on C-8 reverse phase column along with eluants as mixtures of water-acetonitrile-trifluoroacetic acid from 75:25:0.1 to 10:90:0.1 by volume, linear gradient over 13 minutes and 8 minutes of isocratic elution, detection wavelenght = 240 nM), the retention time of title compound (Rt = 10.2 minutes) was the same as the retention time of an authentic non-labelled sample. The ESI mass spectrum showed the protonated molecular ions at m/z 860 amu and also two other characteristic clusters at m/z 877 amu ($M+NH_4^+$) and 882 amu ($M+Na^+$). The NMR spectrum recorded in $CDCl_3$ at 500 MHz showed the following signals expressed as chemical shifts (ppm): 8.24-8.36, m; 7.94-8.04, m; 7.64-7.85, m; 7.33-7.57, m; 6.98, d; 6.30, s; 6.22-6.28, m; 5.78-5.85, dd; 5.68-5.74, d; 4.98, d; 4.96, d; 4.82, dd; 4.39-4.47, m; 4.34, d; 4.24, d; 3.84, d; 3.54, d; 2.53-2.64, m; 2.47, d; 2.41, s; 2.29-2.39, m; 2.27, s; 1.86-1.96, m; 1.83, d; 1.79, s; 1.71, s; 1.27, s; 1,17, s. The isotopic enrichment of 2-[ring-U-^{13}C]-benzoyl-paclitaxel was greater than 99%, as determined by mass spectrometry.

Claims

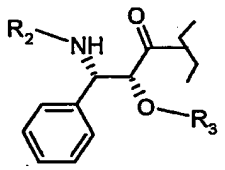
1.A stable labelled taxane of the formula I:



I

wherein R_1 represents hydrogen atom, an acetyl group or a hydroxy protecting group;

R represents hydrogen atom, a hydroxy protecting group or a residue of the formula II:



II

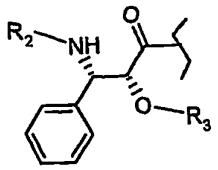
wherein R_2 represents benzoyl or tert-butoxycarbonyl group; R_3 represents hydrogen atom or a hydroxy protecting group;

X, X_1, X_2, X_3 and X_4 independently represent CD, ^{13}CD, ^{13}CH or CH; Y and Y_1 independently represent C or ^{13}C, with the proviso that X, X_1, X_2, X_3, X_4 are not all CH when Y and Y_1 are both C.

2. A stable labelled taxanes of the formula I according to claim 1 in which the hydroxy protecting groups are selected from silyl hydroxy protecting groups and 2,2,2-trichloroethoxycarbonyl group.

3. A stable labelled taxanes of the formula I according to claim 2 in which the hydroxy protecting groups are selected from trimethylsilyl, triethylsilyl, tert-butyldimethylsilyl and siamyldimethylsilyl group.

4. A stable labelled taxanes of the formula I according to claim 1 in which R_1 represents hydrogen atom or an acetyl group, R represents a residue of the formula II



wherein R_2 represents benzoyl or tert-butoxycarbonyl group and
R_3 represents hydrogen atom; X, X_1, X_2, X_3, X_4 are ^{13}CH, Y and Y_1 are both C.

5. A stable labelled taxanes of the formula I which is:

2-[ring-U-^{13}C]-benzoyl-paclitaxel;
2-[ring-U-^{13}C]-benzoyl-docetaxel or
2-[ring-U-^{13}C]-benzoyl-10-desacetyl-baccatin III.

6. Use of a labelled taxane of the formula I as defined in claim 1 in an analytical method.

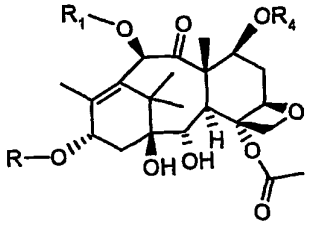
7. Use of a labelled taxane according to claim 6 in order to determine a taxane in the biological fluids.

8. Use of a labelled taxane according to claim 6 as an internal standard.

9. Use of 2-[ring-U-^{13}C]-benzoyl-paclitaxel as an internal standard in an analytical method for determining paclitaxel in the biological fluids.

10. A process for preparing a labelled compounds of the formula I as defined in claim 1, which process comprises:

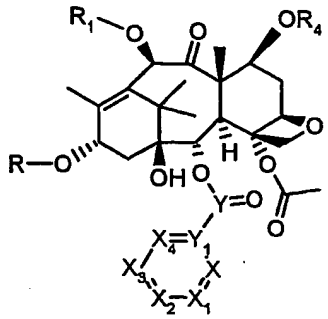
iii) reacting a compound of the formula III



III

wherein R_4 represents a hydroxy protecting group and R, R_1 are as defined in claim 1, with an isotopically labelled benzoic acid,

iv) removing of the hydroxy protecting groups from the resultant compound of the formula IV:



IV

wherein R, R_1, R_4, X, X_1, X_2, X_3, X_4, Y and Y_1 are as defined in claim 1, to give the desired labelled taxanes of formula I and, if desidered,

iii) converting a compound of formula I into a different compound of formula I.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/02634

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D305/14 C07F7/18 G01N33/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D C07F G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 01 94328 A (NAPRO BIOTHERAPEUTICS) 13 December 2001 (2001-12-13) cited in the application page 1; claims	1-3

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 July 2003	28/07/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Francois, J

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 03/02634

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0194328 A	13-12-2001	US 6358996 B1	19-03-2002
		AU 6704301 A	17-12-2001
		CA 2409944 A1	13-12-2001
		EP 1301499 A1	16-04-2003
		WO 0194328 A1	13-12-2001